TISSUE CULTURE IN THE PROPAGATION OF AUSTRALIAN PLANTS

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One of the most exciting fields of plant propagation is tissue culture. This is the process whereby very small pieces of plant tissue such as apical meristems (4, 8), and leaf tips (1), are taken and induced to proliferate into a mass of undifferentiated callus tissue. This undifferentiated callus tissue is then cut up into small pieces, placed on a suitable medium and new plants are formed. Each new plant has the genetic make up and characteristics of the plant the original piece of tissue was taken from. All these processes are carried out in sterile conditions on sterile media in controlled laboratory situations.

A great deal of research is being carried on throughout the world in this field as it has enormous potential as a propagation method. It is particularly important as a rapid method of vegetatively reproducing monocots, which cannot be propagated quickly in great numbers by conventional methods. The method is also very applicable to the rapid clonal multiplication of hybrids, which require some complex seed production systems (2). Already it has become a commercial proposition as a means of propagating orchids, and as a means of producing virus-free carnation plants (3).

Research into the ornamental horticulture of Australian native plants was being carried out at the Canberra Botanic Gardens with particular emphasis on propagation problems. For this reason it was decided to try and develop the technique of tissue culture as a means of growing some plants which were difficult to propagate by other means.

Workers at the University of Western Australia were producing interspecific hybrids of *Anigozanthos* spp., kangaroo paws, (personal communication) and it was decided to develop the tissue culture technique to try and take advantage of these crosses, and others that may eventuate. The ink-spot disease is also a problem on *Anigozanthos* spp and it was possible that some hybrids may show less susceptibility to the disease than others, and this was another reason why a method of vegetative reproduction of these monocots would be valuable.

Anigozanthos flavidus D.C. was chosen for this experimental work, because of its abundance in the Canberra Botanic Gardens, and also its superficial similarity to *Cymbidium* spp. which have proved very easy to propagate by this means.

Initially apical meristems with a 3-5 mm cube of basal tissue below it were dissected from the plant. More recently the first few

leaves surrounding the meristems have been retained. This leaf base tissue is about 8 mm long. These dissected pieces of tissue were sterilised in 95% alcohol for 20 secs., 1% sodium hypochlorite for 20 minutes, then washed in sterile water, and placed on Knudson's C agar, (5) with 15% coconut milk. After about 8 weeks the basal pieces of tissue had turned bright green and had swollen to about one and a half times their original size. This callus tissue was then cut into 4 to 6 pieces, with each cut passing vertically through the meristem. These small pieces of bright green material were placed on fresh Knudson's C and coconut milk agar and were either recut when they had reached a suitable size or allowed to differentiate. Those that were allowed to differentiate, produced shoots and roots after about 12 weeks. Over a period of 8 months approximately 600 plants could have been produced from one meristem had all the pieces been kept.

This is not a great number compared with some other plants, as very little callus tissue was formed. This is consistant with Murashige (6) who also found that it was very difficult to produce callus tissue from apical meristems.

In the last two weeks callus tissue has been successfully grown from parenchyma cells taken from the pith of an unopened flower spike of *A. flavidus*. This was grown on a callus medium for Cymbidium spp (8). There has been insufficient time to determine whether this callus tissue will differentiate to form plants, but the problem of initial rapid callus tissue formation appears to have been solved.

The second species successfully propagated by tissue culture was *Clianthus formosus* (G. Don.) Ford and Vickery — Sturts desert pea, and as far as can be determined was one of the first legumes to be grown by this technique. The work with *C. formosus* began almost by chance.

Seeds of *C. formosus* has been germinated on sterile Knudson's C and coconut milk medium to provide sterile scion material for micrografting. One of the seedlings which had been cut about 10 mm below the cotyledons in preparation for grafting was left in the flask on Knudson's C and coconut milk medium. After about 8 weeks a piece of callus about 7 mm diameter and about 10 mm long was produced. This callus was taken and cut up into tiny pieces (about 2 to 3 mm cubes) and these were placed on Knudson's C and coconut milk medium. After about 3 months shoots and roots were produced.

Because of *C. formosus* callus was so easily obtained it was decided to use other media to try and speed up the various processes. It had been noted that when the callus tissue just began to form on the *C. formosus* seedlings it was white, but after a few weeks in the light it began to turn a very bright green. Callus

taken from the white and green areas responded quite differently. White callus was placed on Murashige and Skoog (7) high salt medium. The callus grew but did not turn green and some tissue became brown. No differentiation had occurred after 4 months. Several other media were tried but all proved unsuccessful. Bright green callus placed on the high salt medium however, produced shoots within 7 days and roots within 8 weeks.

When the bright green callus was placed on high salt medium with 2 ppm naphthaleneacetic acid (NAA) no differentiation occurred, but it remained bright green and increased in size. When this callus was cut up and placed on Murashige and Skoog (7) basal medium, plus kinetin, shoots were produced in about 4 weeks.

All this work was carried out in the light, initially on the laboratory bench with about 400 foot candles of light for 16 hs a day at 22°C. More recently, much better results have been achieved by keeping the cultures in a growth cabinet. The light reading was 2400 foot candles, the day length 20 hours, and the night/day temperatures were 20°C-25°C, respectively.

With both the species tried, plants have been obtained by starting with bright green callus formed in the light. No plants have been obtained when pale callus has been used. The use of bright green callus material seems to be important, but at this stage there does not appear to be a reasonable explanation. It could be that the differentiation of chlorophyll is the first step necessary for further differentiation. The most difficult step in propagation by tissue culture is to induce undifferentiated callus tissue to produce plants. Many workers have been able to obtain callus tissue, and sometimes partial differentiation, but the production of plants has often proved very difficult.

Even though this work is in the early stages it is important to note that plants have been successfully produced from the two species tried. The approach has been simplistic and indeed this may be the key to its success. Undoubtedly tissue culture will play an increasing role in the propagation of plants both commercially and for experimental purposes, and hopefully will find its place in the propagation of Australian plants, particularly where normal methods of vegetative reproduction are difficult or slow.

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THE PRODUCTION OF INTERSPECIFIC HYBRIDS BETWEEN INCOMPATIBLE PAIRS OF EUCALYPTS

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ABSTRACT. A method of breaking interspecific incompatibility barriers between pairs of species in different sections of the genus *Populus* has been found to function in a similar way between pairs of species in some groups of *Eucalyptus*.

INTRODUCTION

Breeding incompatibility is a phenomenon frequently found in flowering plants. It is well known within species and is commonly expressed by the inability of individuals to self fertilize successfully or the inability of some groups of individuals in a species to be fertilized successfully by some other groups within the same species. In a similar way groups of species within larger genera often fail to cross even when other barriers to interbreeding such as geographic isolation or lack of synchronous flowering are removed. Recently considerable attention has been given to the precise mechanisms involved since histochemical and electron microscopic methods have become available to allow much more precise determination of the processes than was previously possible (4). These studies to some extent have already indicated means of approaching the problem of breaking interspecific incompatibility with the consequent opening of ways to extend interspecific breeking in various genera. This allows the consequent production of some forms which may have greater potential utility than those existing.

Work has been carried out with poplar which has lead to the development of techniques for breaking interspecific incompatibility between reproductively isolated species in that genus (5) and it has been found possible to apply the same methods to some extent in eucalyptus.